Posters

Correlated Motions in Proteins

3273-Pos Board B1

Distinct Dynamic Signatures of Amyloidogenic Insulin Revealed by Neutron Spin Echo Spectroscopy

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Insulin is a well-known model protein for studying the formation of amyloid fibrils. At low pH-values, in the presence of sodium chloride (NaCl), and at elevated temperatures, insulin readily aggregates and forms amyloid fibrils. Without NaCl, but in the presence of ethanol, the lag time of this temperature-induced aggregation is increased drastically. In this study, we have analyzed the dynamical properties of bovine insulin following these two aggregation pathways by using neutron spin echo (NSE) spectroscopy. In addition, small-angle X-ray scattering (SAXS) and thioflavin T (ThT) fluorescence experiments were carried out to track the concomitant structural changes of insulin. Measurements have mainly been performed at 318 K, where amyloid fibrils evolve over 25 h, when the insulin solution contains 0.1 mM of NaCl at pD = 2. In contrast, no amyloid fibrils are formed during 25 h at 318 K, when the insulin solution contains ethanol with a volume fraction of 20 % at pD = 2. Remarkably, the NSE data reveal distinct dynamic signatures of insulin under these two solvent conditions. Collective diffusion of insulin molecules can be inferred from an increased diffusion coefficient at low wavevector transfers in the non-fibrillating sample, whereas self-diffusion is observed in the other case. The SAXS data confirm these dynamic behaviors, because a pronounced correlation peak is only observed under conditions of collective diffusion. The dynamic responses of insulin, as revealed here by NSE spectroscopy, are in agreement with intermolecular interaction potentials derived recently from measurements of the static structure factors of insulin and lysozyme.

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Gsatools: Analysis of Allosteric Communication and Functional Local Motions using a Structural Alphabet

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Background

Biomolecular motions play a key role in several biological functions: enzymatic activity, protein-protein interactions, ligand binding and allosteric regulation. Computational approaches, such as Molecular Dynamics (MD), are now routinely used to reproduce the intrinsic dynamics of proteins, but effective tools are still required to gain functional insight from the simulated data.

Methods and Results

We previously suggested a method aimed at recovering the role of local conformational changes in functional motions. To this purpose we developed a Structural Alphabet (SA): a set of 25 canonical states of 4-residue protein fragments (Cα atoms only) describing the most probable local conformations in high-resolution protein structures [1]. The SA provides a mean for the coarse-grained annotation and processing of local conformations in a string format, which lends itself to a range of efficient sequence analysis algorithms. The SA has been successfully used in analysing local changes and allosteric signal transmission [2].

Here we present GSATools [3], a set of SA-related tools interfacing with GROMACS for the analysis of conformational ensembles. GSATools is designed for the investigation of the conformational dynamics of local structures, the functional correlations between local and global motions, and the mechanisms of allosteric communication.

Conclusions

GSATools is a free, easy-to-use and fully documented software for the analysis of conformational ensembles of proteins. The GSATools complement the GROMACS toolkit with a powerful set of analyses to detect, annotate and interpret local motions of functional relevance.

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Recapturing the Correlated Motions of Protein by using Coarse Grained Models

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Long range interaction and allosteric behavior is important for many biological process, such as: metabolic mechanisms, signal transduction pathways. Increasing evidence show that internal dynamics may play an important role in such behavior. Investigating the dynamical effects of proteins is a challenging problem by using all atom molecular dynamics, because these proteins are usually large. Here we use three well-defined coarse grained model: Go, Martini and Cafemol model to examine if these coarse grained model can explore the dynamics effects efficiently, also to explore the effects of mutation. Eglin C is used as a model system. We found that both Go and Cafemol model can recapture the dynamical characteristic of proteins very well, especially for the defined secondary structures, while Martini model explore the dynamics characteristic fairly well. For coil part, three coarse grained models have higher fluctuation and correlated motions than all-atom model. Mutation effects are also discussed.

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Modulation of Active Site Picosecond Dynamics in Mutant Forms of a Thermophilic Alcohol Dehydrogenase (HT-ADH)

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Previous kinetic and solvent-exchange data have suggested that an Arrhenius break observed at 30°C is attributed to a shift in the equilibrium populations of inactive and active microstates accompanied with a change in protein flexibility [1,2]. We employed time-correlated single photon counting to investigate the statistical nature of the fluorescence dynamics as a function of temperature in ht-ADH. Two single-tryptophan variants were used as reporter probes; Trp87 is fortuitously located at the active site and Trp167 is a surface tryptophan >25Å from the catalytic center. The temperature dependence of the time-resolved Stokes shifts reveal that the active site Trp contains highly temperature-dependent relaxation dynamics while the surface Trp is virtually temperature-independent. Though no break is observed that correlates to catalysis, such results are discussed in the context of slower, more global dynamics as a major contributor to catalytic behavior. Using both single-Trp variants, Ala was then introduced to Tyr25 at the dimer interface and Val260 within the cofactor binding site. Such alterations were previously shown to either eliminate or exacerbate the magnitude of the temperature break, respectively [3,4]. The fluorescence dynamics reveal that the temperature-dependent dynamics at Trp87 are drastically altered relative to its wild-type counterpart, while Trp167 shows minor differences. Such results are discussed in the context of an altered conformational landscape that further corroborates an equilibration process occurring more slowly than the ps-timescale.

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ATP Binding Induced Conformational Change in RecQ Helicase

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RecQ helicases are a family of DNA helicases critical for DNA replication, repair, and recombination. These enzymes catalyze DNA strand separation via the binding and hydrolysis of ATP and are essential for resolving complex DNA structures such as double Holliday junctions and D-loops. Mutations in three human RecQ family proteins have been identified in several disorders linked to premature aging and a high predisposition to cancer. Studies of the structural dynamics of RecQ helicases are necessary to fully understand the mechanism of this important class of proteins.

The RecQ family contains a highly conserved core consisting of two RecA-like helicase domains, a zinc binding domain, and a dsDNA-binding winged helix domain. Crystal structures of E. coli RecQ and human RecQ1 indicate that the enzyme binds ATP in the cleft between the two RecA-like domains. This structural detail is consistent with the nucleotide binding motif of other SF2 family helicases, in which ATP hydrolysis drives translocation along ssDNA. However, it is not currently known how the binding and hydrolysis of ATP is coupled to ssDNA translocation in RecQ. Using single molecule fluorescence and molecular dynamics simulations, we have investigated the ATP-dependent dynamics of the core of E. coli RecQ. We show that binding of ATP induces a